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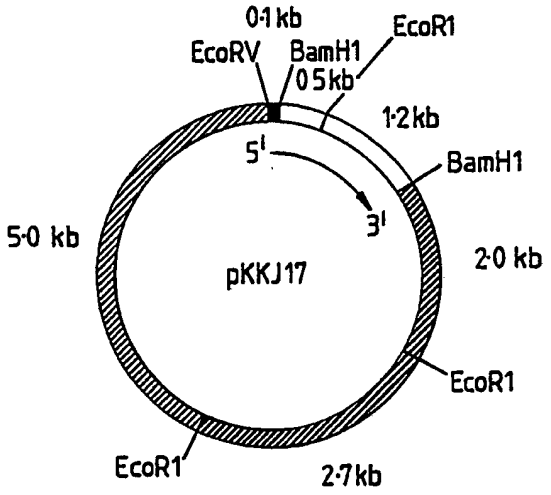

TRAP coding sequence
Baculovirus DNA flanking the polyhedrin gene
pUC sequence
Polyhedrin gene promoter

(57) Abstract

Proteins from the merozoite stage of the malaria parasite, fragments and derivatives thereof, DNA coding for the said proteins, and processes for the preparation of the proteins and plasmid and viral vectors useful in said processes. The invention also provides antibodies to the proteins and immunological compositions containing the proteins.

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POLYPEPTIDES AND DNA ENCODING SAME

This invention relates to polypeptides, and to DNA encoding same, produced by human malaria parasites. It also relates to methods of preparing the polypeptides, to antibodies thereto and compositions for use against malaria.

Plasmodium falciparum malaria is one of the most common infectious diseases in the world today, threatening up to 40% of the world's population. It is a disease of the Third World. There are between 150 and 300 million cases of this disease annually, over 1% of cases are fatal, babies and young children being the most vulnerable. With the advent of insecticides and new parasiticidal drugs developed after World War II it was felt that the disease could be eradicated. The early attempts proved very successful but with time the parasite has developed resistance to drugs such as chloroquine and the mosquito vector (Anopheles) has developed resistance to DDT. As a consequence of this it is necessary to develop new approaches to try to combat the disease. As immunity to the disease develops with increasing age, in endemic areas, a vaccine, together with new anti-malarials and insecticides need to be developed if the disease is to be eradicated.

Current research programmes, throughout the world, are involved in defining what antigens might form part of a useful vaccine. The complex life-cycle of the parasite means that a simple vaccine based on one antigen may not be adequate and that an effective vaccine will probably require antigens from different development stages.

The human malaria parasite, Plasmodium falciparum, has a complex life-cycle, during which different

antigens are produced at particular developmental stages. The major antigen on the sporozoite surface is the circumsporozoite or CS protein, which probably determines the specificity of the interaction between the parasite and liver cells. CS protein contains two conserved amino acid sequences, known as regions I and II, which are separated by a repeating amino acid motif.

The cloning of the gene for this protein has permitted the development of various vaccines. To date vaccine trials using parts of the CS protein have proved disappointing. Immunity to sporozoites does not necessarily prevent the erythrocytic phase of the life-cycle which is associated with clinical disease. Only one sporozoite needs to evade the immune system for clinical disease to occur. Currently CS protein is the only well-characterised protein known to be involved in host-cell recognition. The merozoite is the developmental stage capable of re-infecting fresh red cells. Antibodies which prevent gametocyte differentiation within the mosquito are useful in breaking the transmission cycle as well. Another complexity is the antigenic variation displayed by the parasite. A vaccine against the asexual erythrocytic parasite, need only be partially effective to reduce the severity of the disease. A vaccine against the asexual blood stages of P. falciparum has been developed by Patarroyo et al (Nature Vol.332, 1988, p158) based on the use of synthetic peptides, but this has not proved to be totally effective.

We have now found that polypeptides sharing certain sequence motifs with CS protein are produced during the erythrocytic or merozoite stage of the parasite life-cycle.

Accordingly, the present invention provides a

polypeptide from the group comprising:

a) a polypeptide having the amino acid sequence of Formula I;
b) polypeptides having substantially the same structure and biological activity as a);
c) fragments, derivatives and mutants of a) or b) significantly involved in their biological activity;
d) oligomeric forms of a), b) or c) significantly involved in their biological activity.

It will be understood by those skilled in the art that some variation in structure may occur in naturally occurring biologically active polypeptides and that malarial proteins in particular display antigenic variability. Provided that structural variations do not eliminate the biological activity of interest such as, for example, involvement in parasite recognition of red cells, red cell attachment or merozoite invasion, the present invention includes such variations within its scope.

Thus, although formula I relates to a cloned isolate of P. falciparum from Thailand known as T.9/96, the scope of the invention also includes, for example, a polypeptide derived from another Thailand isolate known as K1. This was known to differ from T9/96 in lacking a Hinf 1 restriction site and having an extra Bgl II site, which has been confirmed by sequencing. Polypeptide from K1 differs from T9/96 in certain details as set out in Table 1 but the conserved regions are intact.

Both T9/96 and K1 are obtainable from the WHO Registry of Standard Strains of Malaria Parasite, Dept. of Genetics, University of Edinburgh, United Kingdom.

Table 1

Comparison of DNA and Polypeptide from T9/96 and K1

Amino acid residue	Nucleotide			Amino Acid	
	Position in codon	T9/96	K1	T9/96	K1
39	1	A	C	S	R
46	1	C	G	Q	E
83	3	T	A	D	E
90	1	G	C	V	L
92	1	G	A	V	I
98	2	A	G	K	R
119	2	G	A	R	K
134	2	C	G	T	S
179	2	G	A	S	N
277	1	A	T	I	L
290	1	T	C	W	R
297	1	G	C	D	H
311	2	C	T	S	F
312	1	T	G	S	A
314	1	C	G	Q	E
337	2	A	G	D	G
341	3	C	A	N	K
359	1	G	C	E	Q
361	1	A	G	K	E
398	2	A	T	H	L
412	1,2	AA	TC	N	S
490	2	A	G	E	G

Generally, about 5% variation in amino acid residues may be tolerated but, as will be understood by those skilled in the art, some regions of the molecule and some residues are more significant than others. Conserved regions which play an important role in biological activity are likely to be less tolerant of variation (e.g. in and around the region displayed for TRAP in Formula III), whereas antigenically important regions, for example around the RGD sequence (residues 307-309 of Formula I) are more subject to variability. TRAP as used herein is an abbreviation for "Thrombospondin related anonymous protein" and indicates one or more of the polypeptides of the present invention. Other regions may be somewhat less significant but there is some evidence of biological activity associated with NP or PN sequences. By "conserved" we mean having significant homology of amino acid residue sequences with other proteins of interest. Thus, for example, the region from about residue 244 to about residue 291 has significant homology with CS proteins from various strains of malaria parasite and with thombospondin and properdin framework proteins as illustrated in Formula III. It is not possible to put precise numerical limits on the degree of homology but 80% or greater say, would in many examples be expected to be signficant.

The present invention also provides fragments of the above polypeptides, preferably containing a conserved sequence, for example, a fragment from the region extending from amino acid residues 244 to 291 of Formula I and more particularly a polypeptide selected from the following group;

a) WDEWSPCSVTCGKGTRSRKR
b) WDEWSPCSVTCGKGTR
c) EWSPCSVTCGKG

d) PCSVTCGKG

e) WSPCSVTCG

The single letters in the formula represent the following naturally occurring L-amino acids: (A) alanine, (C) cysteine, (D) aspartic acid, (E) glutamic acid, (F)phenylalanine, (G) glycine, (H) histidine, (I) isoleucine, (K) lysine, (L) leucine, (M) methionine, (N) asparagine, (P) proline, (Q) glutamine, (R) arginine, (S) serine, (T) threonine, (V) valine, (W) tryptophan, (Y) tyrosine.

Derivatives of the polypeptide of the invention are, for example, where functional groups, such as amino, hydroxyl, mercapto or carboxyl groups, are derivatised, e.g. glycosylated, acylated, amidated or esterified, respectively. In glycosylated derivatives an oligosaccharide is usually linked to asparagine, serine, threonine and/or lysine. Acylated derivatives are especially acylated by a naturally occurring organic or inorganic acid, e.g. acetic acid, phosphoric acid or sulphuric acid, which usually takes place at the N-terminal amino group, or at hydroxy groups, especially of tyrosine or serine, respectively. Esters are those of naturally occurring alcohols, e.g. methanol or ethanol.

Further derivatives are salts, especially pharmaceutically acceptable salts, for example metal salts, such as alkali metal and alkaline earth metal salts, e.g. sodium, potassium, magnesium, calcium or zinc salts, or ammonium salts formed with ammonia or a suitable organic amine, such as a lower alkylamine, e.g. triethylamine, hydroxy-lower alkylamine, e.g. 2-hydroxyethylamine, and the like.

Mutants of the polypeptides of the invention are

characterised in the exchange of one (point mutant) or more, about up to 10, of its amino acids against one or more of another amino acid. They are the consequence of the corresponding mutations at the DNA level leading to different codons.

The present invention also includes within its scope oligomeric forms of the said polypeptides, e.g. dimers and trimers. Such forms may occur naturally and be significant to biological activity. Within the term "oligomeric form" we wish to include both covalently linked molecules and molecules linked by weaker intermolecular bonding, such as hydrogen bonding, into conformationally significant forms.

The present invention also provides DNA sequences coding for the polypeptides of the invention. The DNA sequence coding for the T9/96 strain of P. falciparum is displayed in Formula I but the scope of the present invention extends to variations not affecting the amino acids encoded and also variations such as found in nature and encoding for the K1 strain referred to above, for example.

DNA according to the present invention may be recovered from malaria parasite DNA and genomic libraries by methods known in the art and it will be understood that once the sequence is known direct amplification is possible, by the polymerase chain reaction, for example. (Saiki et al, Science 1985 Vol. 230 pp1350-1354)

The polypeptides of the invention may be prepared by chemical synthesis, where the number of amino acid residues is not too large, or by expression of the appropriate DNA sequences in a host/vector expression system.

Recombinant vectors comprising the appropriate DNA, together with other functional sequences, such as promoter and marker genes, may be made by methods known in the art.

Suitable vectors include recominant plasmids comprising a DNA sequence of the present invention cloned into pUC13 (Pharmacia) or pAc YM1, (Inst. of Virology, Mansfield Road, Oxford, England).

Recombinant viral vectors may be obtained by incorporating the appropriate DNA sequence into viral DNA by methods known in the art. (See, for example, DNA Cloning, Volume II, D.M. Glover, published 1985, IRL Press, Oxford, England.) One suitable method, according to the present invention, involves the combination of a plasmid with a virus using a co-transfection process in a suitable host cell.

Using the plasmid hereinafter identified as pKKJ17, for example, together with the Autographa californica Nucleopolyhedrosis Virus (AcNPV) in Spodoptera frugiperda cells, recombinant virus containing DNA sequence of the present invention may be reproducibly isolated, for example that hereinafter identified as vKKJ17.

According to a further aspect of the present invention we provide antibodies to the polypeptides of the invention. The antibodies may be made by techniques known in the art (see for example: Antibodies, A Laboratory Manual, E. Harlow and D. Lane, Cold Spring Harbour 1988).

The polypeptides of the invention are likely to be useful in the preparation of vaccines and the like against malaria. For this purpose they may be

incorporated as active ingredients in suitable carriers, adjuvants, etc. possibly in combination with other immunologically active materials to provide protection against different stages of the malaria parasite.

Technical and theoretical aspects of the invention will now be discussed for purposes of clarification but it should be understood that the utility of the invention does not depend upon the precise accuracy of this theoretical analysis.

The polypeptides of the invention share certain sequence motifs common to other well-characterised proteins. The most significant homology is based around the sequence WSPCSVTCG, three copies of which have been identified in region I of thrombospondin (TSP), six copies in properdin (P), and one copy in all the circumsporozoite proteins sequenced so far. In addition it shares with certain extracellular glycoproteins, including TSP, the cell-recognition signal (RGD), which has been shown to be crucial in the interaction of several extracellular glycoproteins with the members of the integrin superfamily. Because of their relationship with thrombospondin, the polypeptides of the invention are referred to herein as thrombospondin related anonymous proteins or TRAP proteins. Unlike the CS protein, TRAP proteins are expressed during the erythrocytic stage of the parasite life-cycle.

To search for CS protein-related sequences in the genome of Plasmodium falciparum, an oligonucleotide probe having the sequence ACC.ATT.TCC.ACA.GGT.TAC.ACT.ACA.TGG (shown in Formula IIA), corresponding to region II, was used to probe a genomic Southern blot of Plasmodium falciparum (T9/96) DNA. T9/96 is a cloned isolate of P. falciparum from

Thailand, obtainable from The Dept. of Genetics, Institute of Animal Genetics, Kings Buildings, West Mains Road, Edinburgh, UK. The predicted 9kb Eco RI and 800 bp Bst N1 fragments were detected. The same probe was used to screen two genomic Plasmodium falciparum DNA libraries, one a complete Eco RI digest cloned in lambda-gt 11 and the other a partial Eco RI digest cloned in lambda-gt 10. The true CS protein sequence was not expected to be found in either of these libraries because the two vectors have an upper limit of 8 kb. Several clones were isolated, all of which shared a 2.35 kb Eco RI fragment. The DNA from this fragment as well as that of a neighbouring Eco RI fragment was sequenced (shown in Formula I). The sequence detected by the oligonucleotide, together with the probe sequence, is shown in Formula IIA. There is an open reading frame (Formula I), starting with a methionine residue, which is 559 amino acids long and encodes a protein Mr 63,300. The amino acid sequence includes the conserved nonapeptide Trp-Ser-Pro-Cys-Ser-Val-Thr-Cys-Gly, explaining why the oligonucleotide probe detected this new gene (Formula IIB). This sequence and variations on it are found in TSP, CS protein and properdin. Formulae III illustrate the conserved sequence homology between the TRAP protein of Formula I and CS protein sequences from Plasmodium falciparum (P.f.), P. vivax (P.v.), P. knowlesi (P.k.), P. cynomolgi London strain (P.c.), P. berghei (P.b.), P. yoelii (P.y.), TSP, and the properdin framework. Sequence alignment was achieved using the ALIGN program [Dayhoff et.al., Meth. Enzymol. 91, 524-545, (1983)]. The single letter amino acid code has been used and residues in common with TRAP have been boxed.

The protein sequence of TRAP has two hydrophobic domains at either end of the molecule. The first, at the amino terminal end is probably a signal sequence;

the second, at the carboxy terminus, resembles a transmembrane sequence. There is a cluster of cysteine residues around and including the conserved amino acid sequence suggestive of a secondary structure formed from intermolecular or intramolecular disulphide bonds. Cysteine residues occur in similar positions around the conserved regions in CS protein, TSP and properdin. Evidence for such secondary structure is provided by the fact that antibodies raised against the CS derived peptide containing region II gave poor reaction to both native and denatured CS protein, suggesting a highly ordered configuration [Ballou et.al., Science 228, 996-999, (1985)]. Beyond the conserved region the sequence becomes rich in proline but this does not form part of a repeat characteristic of many other malaria proteins. Submerged within this sequence is an RGD motif (amino acids 307-309), which is characteristic of many glycoproteins involved in cell recognition. TRAP is the first malarial protein to have this motif. TSP has such an RGD motif as well as an IQQ motif which has been implicated in cross-linking to Factor $XIII_a$; TRAP also has an IQQ motif (amino acids 76-78). There are four possible sites for N-glycosylation. Like most malarial antigens, the amino acid composition is unusual in that it is particularly rich in asparagine and proline.

The CS protein gene is only expressed during the sporozoite stage of the life-cycle of the malaria parasite. A different protein (CRA or Ag 5.1), expressed in asexual parasites, bears an epitope which is cross-reactive with monoclonal antibodies directed against the NANP repeat structure in CS protein. Sequence data for this protein reveals an area of homology to $(NANP)_2$ and no other sequence characteristic of the CS protein.

Northern blot analysis using a CS gene probe did not detect any RNA species from erythrocytic stages. Similar analysis (see Figure 1C) using a TRAP gene (of Formula I) probe showed that RNA species of about 20S were detected in the two isolates examined, ITO and FCR3A2, indicating that the TRAP gene is expressed during the erythrocytic stage of the life-cycle but not in EBV-transformed lymphocytes, indicating that the TRAP probe was not detecting human sequences present in blood due to contamination and is therefore parasite-specific. The size of the RNA transcript is compatible with it coding for a protein of Mr 63,300. Antibodies have been raised to TRAP beta-galactosidase fusion proteins. They react on Western blots with a protein of about 65 kd (the predicted size for the TRAP gene product) as well as a number of other parasite proteins including mature infected erythrocyte surface antigen (MESA) and 332. Further examination of the deduced amino acid sequence for TRAP reveals several motifs centred around a Glu-Glu (E-E) motif, and this probably explains this cross-reaction. Indirect immunofluorescence suggests that TRAP is synthesised during the final stages of schizogony.

The occurrence in two both vertebrate and invertebrate stages of Plasmodium falciparum of a highly conserved motif which is also present in thrombospondin and in properdin suggests that TRAP proteins might be of functional significance. A possible role for the CS protein of sporozoites is recognition and entry into hepatocytes. Synthetic peptides from regions I bind specifically to hepatocytes in vitro; such studies have not yet been reported for region II. Two other parasite-cell interactions are critical in the life cycle of Plasmodium falciparum. Its virulence is related to its propensity to sequester in deep vascular beds. This process, which depends on the interaction of

parasite-induced modifications on the red cell surface with receptors on endothelial cells involves thrombospondin. Both thrombospondin itself and thrombospondin antibodies inhibit the cytoadherence of infected red blood cells in in vitro models of sequestration. This, taken together with the evidence that implicates platelet glycoprotein IV (the thrombospondin receptor) as having a crucial role in cytoadherence would be consistent with the presence on the infected erythrocyte of a parasite-induced thrombospondin analogue. The cytoadherence antigen Pf EMP I is thought to be 300 kd; this does not exclude TRAP as there is some evidence that cytoadherence involves parasite modification of a host protein and TRAP could fulfil this role.

The other parasite-cell interaction is the recognition and invasion of red cells by free merozoites. If TRAP were to be present on the free merozoite surface the homology with properdin, which binds to C3b, might play a role in the recognition of C3b or its breakdown products on the red cell surface. The closely related parasite Babesia rhodiani has developed a strategy for entering erythrocytes involving C3b. The observation that entry of red cells by Plasmodium falciparum merozoites does not require serum complement components does not exclude the involvement of complement components already on the red blood cell surface.

Examples of the invention and other technical details will now be described with reference to the accompanying formulae and drawings in which:

Formula I represents the amino acid residue sequence of a polypeptide according to the present invention, using the single letter amino acid

code, and the nucleotide sequence of a DNA coding therefore, together with flanking non-coding sequences. Nucleotides are numbered from 1 to 3102 and amino acid residues from 1 to 559 corresponding to coding nucleotides from 312 to 1989;

Formula IIA shows a comparison of a 27-base oligonucleotide probe and a DNA sequence detected by it from a lambda gt11 genomic library of P. falciparum and

Formula IIB is a comparison of amino acid and nucleotide sequences complementary to the probe sequences;

Formulae III show a comparison of amino-acid sequences around and including the conserved nonapeptide motif of the polypeptide of Formula I with other proteins;

Figure 1A represents a Southern blot analysis of DNA from a cloned line T9/96 of P. falciparum hybridised with the polypeptide of Fig. 1, used as a probe;

Figure 1B is similar to figure 4A but hybridised with a CS protein probe;

Figure 1C represents a Northern blot analysis of total RNA from EBV transformed lymphocytes and from two P. falciparum isolates;

Figures 2 and 3 are diagramatic representations of plasmids pKR5 and pKKJ17 respectively as provided by the invention showing restriction sites and other features.

Referring to Formulae IIA and IIB, one 27-base oligonucleotide sequence was synthesised from the amino acid sequence PCSVTCGNG in region II of CS protein. The complementary strand was synthesised so that both DNA and RNA sequences could be detected. The oligonucleotide was synthesised on an Applied Biosystems model 308A (Trade Mark) synthesizer by monomer addition of phosphoramidites to a solid support. The de-protected probe was purified by preparative polyacrylamide gel electrophoresis. The end-labelled oligonucleotide was used to screen 4 x 10^5 plaques (on E.coli Y1088) of an Eco RI digest of P. falciparum T9/96 DNA cloned into the Eco RI site of lambda-gt11. Six positive plaques were identified which rescreened. These contained 2.35 kb Eco RI fragments. Hybridisation was carried out for 16 hours at 37°C in 6 x NET (1 x NET = 0.15 M NaCl, 0.015 N tris-HCl pH 8.0, 1 mM EDTA), 5 x Denhardt's, 0.5% NP40, 100 micrograms/ml sheared salmon sperm DNA. Washing was done at 37°C in 6 x SSC (1 x SSC = 0.15 M NaCl, 0.015 M sodium citrate) 0.1% SDS, followed by a one minute wash in the same solution at 60°C. The filters were subjected to autoradiography for 16 hours with pre-flashed X-ray film.

The 2.35 kb Eco RI insert from the lambda-gt11 phage was subcloned into pUC8 and M13mp10. Dideoxy sequencing of this fragment revealed an incomplete open reading frame. A second library was constructed by cloning a partial Eco RI digest of T9/96 into lambda-gt10, and was screened with the original 2.35 kb fragment. 1.1 x 10^5 plaques (on E.coli NM514) were screened and clones with overlapping sequences were obtained. The complete DNA sequence of the TRAP gene and its flanking sequences were established using the chain termination procedure of Sanger et.al.[J.Mol. Biol., 143, 161-178 (1980)]. The information was

obtained from 'shotgun' clones in M13mp10 which enabled both strands to be sequences. Gaps were filled in using custom-synthesised oligonucleotide primers. This approach proved the most efficient way of deriving the DNA sequence where there was an absence of restriction enzyme sites; a particular problem in regions of A-T rich DNA. The DNA sequence handling programmes of Staden were used to analyse the data [Nucl. Acids Res. 10, 4731-4751 (1982)]. The site to which the oligonucleotide probe hybridised is underlined in Formula I. The asparagine residues which are potential sites for N-linked glycosylation have asterisks. The RGD sequence (residues 307-309) is overlined. The IQQ motif (residues 76-78) is boxed.

Referring to Figs. 1A and 1B, (Southern blots) lanes 1-11 correspond to restriction digests with the following enzymes, Asp 718, Bam HI, Bgl II, EcoRI, Hinc II, Hind III, Taq I, Bst NI, Hha I, Hpa II and Msp I respectively.

In Fig. 1C, (Northern blots) lane 1 corresponds to EBV transformed lymphocytes (25 μg); lane 2, P. falciparum isolate ITO (Brazilian strain) (25 μg) and lane 3 P. falciparum FCR3 A2 (Rockerfeller strain) (cloned) (25 μg).

Southern blotting and hybridisation was carried out as described by Woo et al. [Nature 306, 151-155 (1984)]. Northern blotting was as described by Robson et al. [Proc. Natl. Acad. Sci. USA, 79, 4701-4705 (1982)]. Hybond-N (Trade Mark) membranes were used to permit re-hybridisation of the same filter. The filter in panel A was initially hybridised with the two Eco RI fragments corresponding to the TRAP sequence; the signal was removed and the filter rehybridised with that containing the CS protein gene sequence. The probe in

panel C was the same as in panel A. The filter in panel C was also hybridised to the same probe as panel B: no hybridisation signal was observed.

In order to produce larger quantities of the polypeptides and DNA of the invention, in purer form the following procedures were followed.

Polycolonal antibodies to several purified beta-galactosidase TRAP fusion proteins were generated. Purification of the proteins involved denaturation by detergent and poly acrylamide gel electrophoresis so that any important conformational epitopes were destroyed. As these antibodies also recognised other parasite antigens on Western blots they could not be used to purify native TRAP from cultured parasites. It was therefore decided to pursue an alternative strategy using eukaryotic expression vectors designed to produce large quantities of native protein which should be correctly processed and easily purified.

One possible expression system utilises baculoviruses. The baculovirus expression system allows high level expression of foreign genes in a eukaryotic environment. It takes advantage of natural gene regulation, that is the very late but highly abundant expression of the polyhedrin gene. Owing to the large size of baculovirus genomes, most recombinant virus construction relies on in vivo recombination to replace a viral allele with the gene of interest. Transplacement plasmids contain the site for foreign gene insertion as well as flanking viral sequences that provide homologous sequences for recombination. Co-transfection of cells with viral and recombinant plasmid DNAs allows cell-mediated allelic replacement of the target viral gene with the plasmid-borne foreign gene construct.

For foreign genes to be expressed abundantly in the baculovirus expression system they need to be engineered to lack introns as well as the majority of 5' and 3' flanking sequences. TRAP genes do not contain introns and using the polymerase chain reaction (PCR) we have engineered the TRAP gene of Fig. 1 with a complete open reading frame as a Bam HI restriction fragment and cloned into the transfer vector pAcYM1 (obtainable from the Institute of Virology, Oxford, England).

This is carried out as follows. Two oligonucleotide primers were designed containing Bam HI sites as well as the 5' and 3' ends of the TRAP genes. The sequences of these two primers are:-

A GGATCCAAAATAATGAATCATCTTGGG

B GGATCCGTATTATATTTAATTCCACTCG

the underlined sequences correspond to the ends of the coding sequence. In primer A this is the coding strand and in primer B this is the complementary strand; this is necessary because DNA is always synthesised in the 5' to 3' direction. The DNA amplified was a genomic subclone of T9/96 DNA in M13. The reaction was set up as described by Saiki et al (Science, 1985, Vol. 230, pp1350-1354) utilising Taq 1 polymerase [(1 mM) oligonucleotide primers A and B, 10 mM deoxynucleoside triphosphates and 1 ng of RV9 (the subclone of TRAP in M13)]. The cycle times and temperatures were 1' at 93°C, 1'37°C,5' at 72°C, the number of cycles was 15. After amplification the complete reaction mix was extracted with phenol/$CHCl_3$, chloroform, followed by gel filtration to remove the unused deoxynucleoside triphosphates. The ends of the DNA product were repaired using the Klenow fragment of deoxyribonuclease 1 and fresh deoxynucleoside triphosphates. The reaction

was again terminated by phenol/$CHCl_3$ extraction and gel filtration using Sephadex G50/80. The 5' ends of the DNA were phosphorylated using T4 polynucleotide kinase and adenosine triphosphate. The reaction was terminated by phenol/$CHCl_3$ extraction and the DNA recovered by propan-2-ol precipitation. This material was used as the substrate of a ligation reaction using T4 DNA ligase and phosphatased Sma 1 cut pUC13 (Pharmacia). Constructs containing the desired insert were obtained and their authenticity checked by DNA sequencing. One such plasmid was pKR5. The fragment could be released from pUC13 by Bam H1 digestion demonstrating that the necessary restriction sites would permit transfer of the transplacement plasmid pAcYM1. pKR5 was digested with Bam HI and Hae III, the reaction terminated by phenol/$CHCl_3$ extraction, followed by ethanol precipitation. This digest was carried out to prevent gel purification of the desired 1.7 kb Bam HI fragment prior to religation into the Bam HI site of pAcYMI. A similar ligation and transformation was carried out with the insert and the new vector pAcYMI. Again constructs containing the desired fragment were obtained. The orientation of the insert containing the TRAP sequence relative to the polyhedrin promoter was checked by restriction mapping and sequencing. This construct has been called pKKJ17 and the map is shown in Fig. 3. A deposit of this plasmid has been made at the National Collections of Industrial and Marine Bacteria Ltd. (NCIMB), Torrey Research Station, P.O. Box 31, 135 Abbey Road, Aberdeen, AB9 8DG, United Kingdom on 14th July, 1989 under Accession Number NCIMB 40,164.

Using pKKJ17, we were able to set up a transfection of Spodoptera frugiperda cells as outlined below. 25 μg of pKKJ17 together with 1 μg of caesium chloride purified Autographa californica Nucleopolyhedrosis Virus (AcNPV) DNA was prepared in Hepes buffered saline pH 7.5

containing 10 mM glucose and 125 mM $CaCl_2$. A calcium/DNA complex was allowed to form over a 45 minutes period prior to addition to freshly plated Spodoptera frugiperda cells (1.5×10^6 cells) and incubation for 1 hr at room temperature. The DNA precipitate was removed and fresh medium (TC100) added and the cells incubated at 20°C until a cytopathic effect had been observed (3 days post transfection). The viruses produced by these cells were both wild type AcNPV and recombinant AcNPV containing the TRAP gene. Plaque purification following titration permitted the isolation of pure recombinant virus. This was facilitated by the fact that wild type AcNPV had intact polyhedrin gene and so produced occluded plaques whereas recombinants did not. The differences between the two types of plaque can be visualised using light microscopy.

After the first transfection one recombinant plaque was seen and purified. Approximately one in two hundred plaques were recombinant provided that caesium chloride purified viral DNA was used in earlier stages. Recombinant virus was identified as vKKJ17 and a deposit has been made on 14th July, 1989 at European Collection of Animal Cell Cultures (ECACC), Public Health Service Laboratory, Centre for Applied Microbiology and Research, Porton Down, Salisbury, Wiltshire, SP4 OJG, United Kingdom, under Accession number 89071402.

AcNPV is the prototype virus of the family Baculoviridae. During infection of Spodoptera frugiperda cells with this virus two types of virus progeny are produced, extracellular virus particles and occluded virus particles. The occluded virus particles are embedded in proteinaceous material, comprising the polyhedrin protein. Under the microscope these are visible as polyhedra. These viral inclusions are an important part of the life cycle as it is through

ingestion of these particles that insect larvae become infected.

The recombinant virus vKKJ17 lacks the polyhedrin gene and so cannot make occluded particles. The polyhedrin gene has been replaced by the TRAP gene. As a onsequence the virus vKKJ17 remains infectious for the tissue culture system (Sp. frugiperda) but cannot infect insect larvae. The expression of the TRAP protein is under the control of the polyhedrin protein. This means that TRAP protein starts to be synthesized 20 hr post infection. The polyhedrin protein is the most adundant protein in the wild-type virus and generally foreign genes under the expression of the polyhedrin promoter produce high levels of the protein of interest. In vKKJ17 the TRAP sequence is on a Bam Hl fragment which replaces all of the polyhedrin coding sequence.

Baculoviruses are double stranded DNA viruses, where the DNA is circular. The size of the DNA is 135 kb.

Production of TRAP was tested by infecting Sp. frugiperda cells with recombinant virus vKKJ17 and growing the cells for 72 hrs before SDS-polyacrylamide gel electrophoresis and Western blotting. The antibody used in the detection system was rabbit polyclonal produced using the beta-galactosidase fusion material. This recombinant virus did in fact synthesize TRAP polypeptide according to the present invention. Further analysis demonstrated that this material was secreted into the culture supernatant. This factor aided purification.

At high multiplicity of infection there is no requirement to maintain Sp. frugiperda cells in medium containing serum. Advantage was taken of this fact so

cells were infected at high multiplicity of infection with vKKJ17 and incubated at 28°C in the absence of serum for 72 hrs. The infected cells were removed by centrifugation (Beckman JA10 4K, 10' 4°C).

The clarified supernatant containing TRAP and virus particles was concentrated by $(NH_4)_2SO_4$ precipitation followed by dialysis. The virus particles were removed by centrifugation (Beckman JA20, 20K, 4°C, 90'). This material was subjected to ion exchange chromatography (Mono Q, Pharmacia, LKB). Fractions containing TRAP were concentrated by lyophilisation prior to further purification by gel filtration (TSK G3000, Pharmacia, LKB). TRAP was greater than 30% pure possibly 90%, an amount of degradation had occurred and this is the reason for the inaccuracy in this estimate.

Insect cells apparently recognise and cleave mammalian signal sequences which direct proteins to the endoplasmic reticulum (ER). It also appears that sites which are targeted for glycosylation in insect cells (Sp. frugiperda) are the same as those for mammalian cells. The insect cells appear to lack galactose and sialic acid transferases and therefore cannot form 'complex' oligosaccharides, this results in trimming of the N-link to a central core of $Man_3GlcNAc_2$. Nevertheless, proteins such as interleukins and interferons made using these systems have been shown to be biologically active. TRAP produced in this manner appears to have been assembled into a dimer or trimer indicating that oligomeric forms of the polypeptide are functional.

Complementary work was carried out to explore possible variation in the DNA sequence which results in amino acid sequence. The polymerase chain reaction was again employed using oligonucleotide primers A and B.

The substrate for the enzymatic reaction has been total genomic DNA from various geographical isolates. The amplified material was treated in a similar fashion prior to cloning into the Sma 1 site of the sequencing vector M13mp8 (Amersham). Using a series of nested primers it was possible to sequence the complete 1.7 kb insert for K1 (a Thai isolate different from T9/96). Some differences from the sequence of Formula I were found, as displayed in Table 1, but the conserved regions were all intact. Single base substitutions result in 22 amino acid substitutions. There is one single substitution which does not lead to an amino acid change. This maintains a cysteine residue. These sorts of results have been observed in other antigenically variable proteins involved in cell attachment screened, as well as in CS protein.

Oligonucleotide sequence	ACC	ATT	TCC	ACA	GGT	TAC	ACT	ACA	TGG
		*	*						
Sequence detected	ACC	TTT	ACC	ACA	AGT	TAC	ACT	ACA	TGG

FORMULA 2A

CS protein									
Amino acid sequence	P	C	S	V	T	C	G	N	G
DNA sequence	CCA	TGT	AGT	GTA	ACT	TGT	GGA	AAT	GGT
TRAP									
Amino acid sequence	P	C	S	V	T	C	G	K	G
DNA sequence	CCA	TGT	AGT	GTA	ACT	TGT	GGT	AAA	GGT

FORMULA 2B

```
   1 taaaaatattctttgtttatattaagatatttaactttactatacacgtaatttttgtaatatatatatatatat
  76 gtatattataggaagaacgtctaatatacataacattgttcttatatataatatatttgattgtgaatttatata
 151 catttatatgatatcacaccaaataaattacacttatatcaaatatacctgtgtatataaatatatgattatata
 226 atttgtatgtgcatgcgtacaagaaaatattatcttttaaggtataatatatttgttttagtattattattaaaa
                1                                                  11
               M   N   H   L   G   N   V   K   Y   L   V   I   V   F   L   I
 301 ttgtaaaaata ATG AAT CAT CTT GGG AAT GTT AAA TAT TTA GTC ATT GTG TTT TTG ATT
                   21                                          31
     F   F   D   L   F   L   V   N   G   R   D   V   Q   N   N   I   V   D   E
 360 TTC TTT GAT TTG TTT CTA GTT AAT GGT AGA GAT GTG CAA AAC AAT ATA GTG GAT GAA
                       41                                          51
     I   K   Y   S   E   E   V   C   N   D   Q   V   D   L   Y   L   L   M   D
 417 ATA AAA TAT AGT GAA GAA GTA TGT AAT GAT CAG GTA GAT CTT TAC CTT CTA ATG GAT
                           61                                          71
     C   S   G   S   I   R   R   H   N   W   V   N   H   A   V   P   L   A   M
 474 TGT TCT GGA AGT ATA CGT CGT CAT AAT TGG GTG AAC CAT GCA GTA CCT CTA GCT ATG
                               81                                          91
     K   L   I   Q   Q   L   N   L   N   D   N   A   I   H   L   Y   V   N   V
 531 AAA TTG ATA CAA CAA TTA AAT CTT AAT GAT AAT GCA ATT CAC TTA TAT GTT AAT GTT
                                   101                                         111
     F   S   N   N   A   K   E   I   I   R   L   H   S   D   A   S   K   N   K
 588 TTT TCA AAC AAT GCA AAA GAA ATT ATT AGA TTA CAT AGT GAT GCA TCT AAA AAC AAA
                                       121
     E   K   A   L   I   I   I   R   S   L   L   S   T   N   L   P   Y   G   R
 645 GAG AAG GCT TTA ATT ATT ATA AGG TCA CTC TTA AGT ACA AAT CTT CCA TAT GGT AGA
     131 *                                 141
     T   N   L   T   D   A   L   L   Q   V   R   K   H   L   N   D   R   I   N
 702 ACA AAC TTA ACT GAT GCA CTG TTA CAA GTA AGA AAA CAT TTA AAT GAC CGA ATC AAT
         151                                   161
     R   E   N   A   N   Q   L   V   V   I   L   T   D   G   I   P   D   S   I
 759 AGA GAG AAT GCT AAT CAA TTA GTT GTT ATA TTA ACA GAT GGA ATT CCA GAT AGT ATT
             171                                   181
     Q   D   S   L   K   E   S   R   K   L   S   D   R   G   V   K   I   A   V
 816 CAA GAT TCA TTA AAA GAA TCA AGA AAA TTA AGT GAT CGT GGT GTT AAA ATA GCT GTT
                 191                                   201
     F   G   I   G   Q   G   I   N   V   A   F   N   R   F   L   V   G   C   H
 873 TTT GGT ATT GGA CAA GGT ATT AAT GTA GCT TTC AAC AGA TTT CTT GTA GGT TGT CAT
                     211                                   221
     P   S   D   G   K   C   N   L   Y   A   D   S   A   W   E   N   V   K   N
 930 CCA TCA GAT GGT AAA TGT AAC TTG TAT GCT GAT TCT GCA TGG GAA AAT GTA AAA AAT
                         231                                   241
     V   I   G   P   F   M   K   A   V   C   V   E   V   E   K   T   A   S   C
 987 GTT ATC GGA CCC TTT ATG AAG GCT GTT TGT GTT GAA GTA GAA AAA ACA GCA AGT TGT
                             251                                   261
     G   V   W   D   E   W   S   P   C   S   V   T   C   G   K   G   T   R   S
1044 GGT GTT TGG GAC GAA TGG TCT CCA TGT AGT GTA ACT TGT GGT AAA GGT ACC AGG TCA
                                 271                                   281
     R   K   R   E   I   L   H   E   G   C   T   S   E   I   Q   E   Q   C   E
1101 AGA AAA AGA GAA ATC TTA CAC GAA GGA TGT ACA AGT GAA ATA CAA GAA CAA TGT GAA
                                     291                                   301
     E   E   R   C   P   P   K   W   E   P   L   D   V   P   D   E   P   E   D
1158 GAA GAA AGA TGT CCT CCA AAA TGG GAA CCA TTA GAT GTT CCA GAT GAA CCC GAA GAT
                                         *   311
     D   Q   P   R   P   R   G   D   N   S   S   V   Q   K   P   E   E   N   I
1215 GAT CAA CCT AGA CCA AGA GGA GAT AAT TCT TCT GTC CAA AAA CCA GAA GAA AAT ATA
     321                                     331
     I   D   N   N   P   Q   E   P   S   P   N   P   E   E   G   K   D   E   N
1272 ATA GAT AAT AAT CCA CAA GAA CCT TCA CCA AAT CCA GAA GAA GGA AAG GAT GAA AAT
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FORMULA I

(continued on p25)

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                341                                                 351
      P   N   G   F   D   L   D   E   N   P   E   N   P   P   N   P   D   I   P
1329 CCA AAC GGA TTT GAT TTA GAT GAA AAT CCA GAA AAT CCA CCA AAT CCA GAT ATT CCT
                361                                                 371
      E   Q   K   P   N   I   P   E   D   S   E   K   E   V   P   S   D   V   P
1386 GAA CAA AAA CCA AAT ATA CCT GAA GAT TCA GAA AAA GAA GTA CCT TCT GAT GTT CCA
                381                                                 391
      K   N   P   E   D   D   R   E   E   N   F   D   I   P   K   K   P   E   N
1443 AAA AAT CCA GAA GAC GAT CGA GAA GAA AAC TTT GAT ATT CCA AAG AAA CCC GAA AAT
                401                                                 411
      K   H   D   N   Q   N   N   L   P   N   D   K   S   D   R   N   I   P   Y
1500 AAG CAC GAT AAT CAA AAT AAT TTA CCA AAT GAT AAA AGT GAT AGA AAT ATT CCA TAT
                421                                                 431
      S   P   L   P   P   K   V   L   D   N   E   R   K   Q   S   D   P   Q   S
1557 TCA CCA TTA CCT CCA AAA GTT TTG GAT AAT GAA AGG AAA CAA AGT GAC CCC CAA AGT
                441                                                 451
      Q   D   N   N   G   N   R   H   V   P   N   S   E   D   R   E   T   R   P
1614 CAA GAT AAT AAT GGA AAT AGG CAC GTA CCT AAT AGT GAA GAT AGA GAA ACA CGT CCA
                *   461                                             *   471
      H   G   R   N   N   E   N   R   S   Y   N   R   K   Y   N   D   T   P   K
1671 CAT GGT AGA AAT AAT GAA AAT AGA TCA TAC AAT AGA AAA TAT AAC GAT ACT CCA AAA
                                481                                                 491
      H   P   E   R   E   E   H   E   K   P   D   N   N   K   K   K   G   E   S
1728 CAT CCT GAA AGG GAA GAA CAT GAA AAG CCA GAT AAT AAT AAA AAA AAA GGA GAA TCA
                                501
      D   N   K   Y   K   I   A   G   G   I   A   G   G   L   A   L   L   A   C
1785 GAT AAT AAA TAT AAA ATT GCA GGT GGA ATA GCT GGA GGA TTA GCT TTA CTC GCA TGT
511                             521
      A   G   L   A   Y   K   F   V   V   P   G   A   A   T   P   Y   A   G   E
1842 GCT GGA CTT GCT TAT AAA TTC GTA GTA CCA GGA GCA GCA ACA CCC TAT GCC GGA GAA
        531                                                 541
      P   A   P   F   D   E   T   L   G   E   E   D   K   D   L   D   E   P   E
1899 CCT GCA CCT TTT GAT GAA ACA TTA GGT GAA GAA GAT AAA GAT TTG GAC GAA CCT GAA
            551
      Q   F   R   L   P   E   E   N   E   W   N
1956 CAA TTC AGA TTA CCT GAA GAA AAC GAG TGG AAT taaatataatacttgaataatattttatgta
2020 atatagtgaaatacacaacaacaaaaaaaaaaaaaaataataaaataaaataaaataaataaataaaataa
2095 aataaaagataaaaataataaaaaaataataatagtaaggataaaaaagaataatatgaaaaatacaaatatata
2170 tttgtatataataaaactcttataaaaattatacataatagtagcaagagaaataagattgtgttaattctttat
2245 tttattttatttttatttgtttctttttttctttttttctttttttctttttttctttttttttttttttttt
2320 ttttttagtaaatcgctatatataatctattttatttactcggtaatttgttttatttttttttttattttttta
2395 ttatttgttgattacttttatgttttattctctctttaatttataatcatttatatatcactaatgaatgtgtta
2470 ttatatataatataaattcgccatgtttttatttttcttcatatatttatttttgttaatttaaacattttagtaga
2545 ataaaagaaacctataataaaaaatttcataataacaaaaattacaaaaaaaaaaaaaaaaaaaaaaaataataa
2620 taataataataataataaatcctcatcttaacatacatatttataataatatatagaaaaaatttttcctata
2695 taaaatttaaaatgaaaaaatatatattaaactaattaagaaaaaaattaagaatacatatataaatatatataa
2770 attatatatatatatatattaaaatatgaattacacaaatttttgtgtcactaacttttagaacaaaataaata
2845 taaaaaaaaaactttacaaattgtgtctaagaaattaaattatttttaattaacaaaatattgattcgaaaacaaa
2920 ttgtggaatttttaaaaaatcatcaattatttttgactcccgtttttcatcattttgttcattatccttatgatc
2995 tttatttttaatatttaatttttcacatagaactttagaaaattcataatcgcatatttgaaaatttgtatttaa
3070 atcttcatttataatttcattaacatagaattc
```
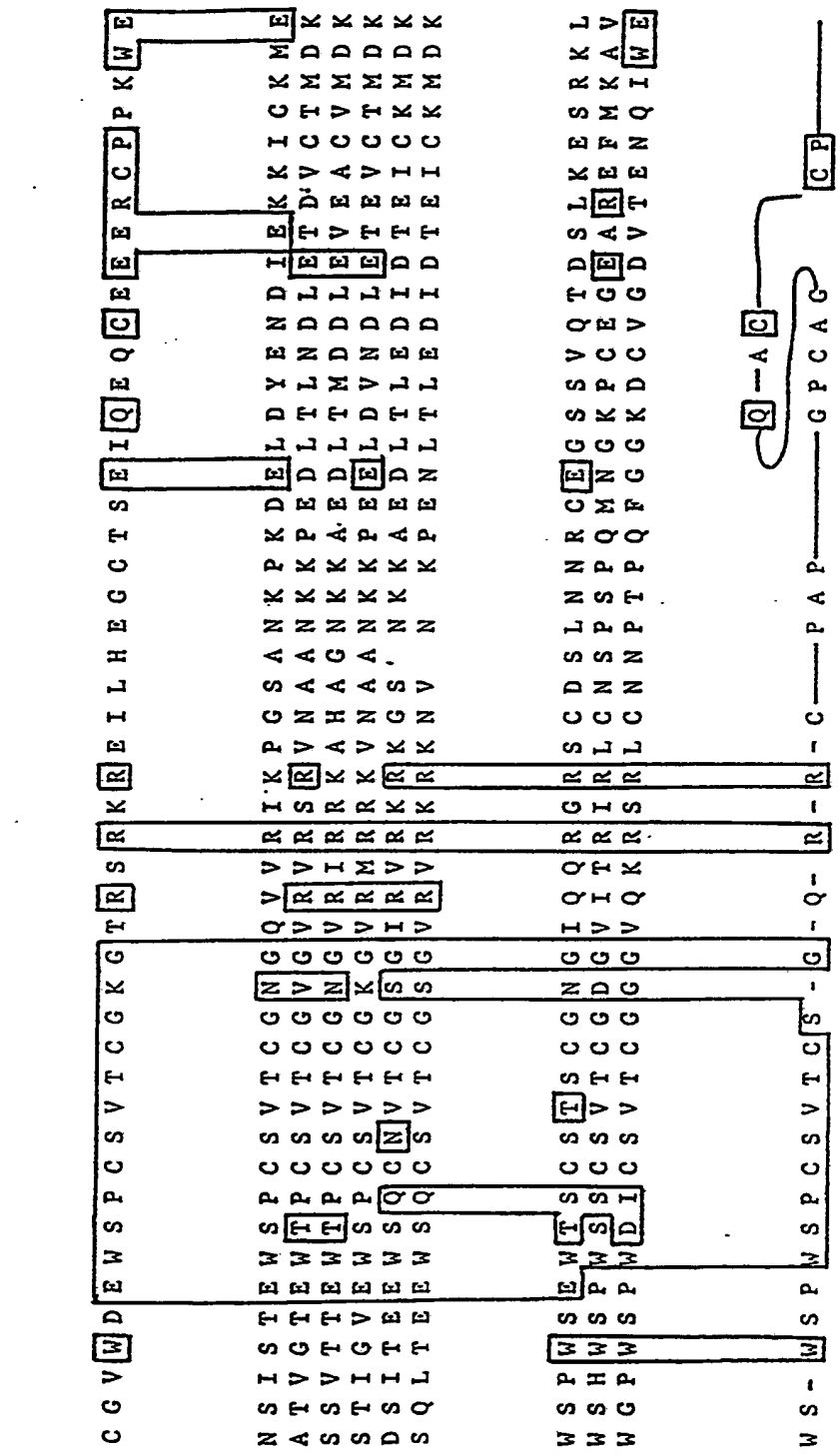

FORMULA I

(continued from p24)



FORMULAE III

ANNEX M3

International Application No: PCT/ /

MICROORGANISMS

Optional Sheet in connection with the microorganism referred to on page 8, line 17 of the description [1]

A. IDENTIFICATION OF DEPOSIT [2]

Further deposits are identified on an additional sheet ☒ [3]

Name of depositary institution [4]

The National Collections of Industrial and Marine Bacteria Ltd.

Address of depositary institution (including postal code and country) [4]

Torrey Research Station, P.O. Box 31, 135 Abbey Road, Aberdeen, AB9 8DG, United Kingdom.

Date of deposit [5]	Accession Number [6]
14th July, 1989 (14.07.89)	NCIMB 40164

B. ADDITIONAL INDICATIONS [7] (leave blank if not applicable). This information is continued on a separate attached sheet ☐

In respect of those designations in which a European patent is sought, a sample of the deposited micro-organism will be made available until the publication of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample.
(Rule 28(4) EPC).

C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE [8] (if the indications are not for all designated States)

D. SEPARATE FURNISHING OF INDICATIONS [9] (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later [9] (Specify the general nature of the indications e.g., "Accession Number of Deposit")

E. ☐ This sheet was received with the international application when filed (to be checked by the receiving Office)

(Authorized Officer)

☐ The date of receipt (from the applicant) by the International Bureau [10]

was

(Authorized Officer)

(January 1985)

Form PCT/RO/134 (January 1981)

ANNEX M3

International Application No: PCT/ /

MICROORGANISMS

Optional Sheet in connection with the microorganism referred to on page 8, line 23 of the description [1]

A. IDENTIFICATION OF DEPOSIT [2]

Further deposits are identified on an additional sheet [X] [3]

Name of depositary institution [4]

European Collection of Animal Cell Cultures (ECACC)

Address of depositary institution (including postal code and country) [4]

Public Health Service Laboratory, Centre for Applied Microbiology and Research, Porton Down, Salisbury, Wiltshire, SP4 OJG, United Kingdom.

Date of deposit [5]	Accession Number [6]
14th July, 1989 (14.07.89)	89071402

B. ADDITIONAL INDICATIONS [7] (leave blank if not applicable). This information is continued on a separate attached sheet [ ]

In respect of those designations in which a European patent is sought, a sample of the deposited micro-organism will be made available until the publication of the grant of the European patent or until the date on which the application has been refused or withdrawn or is deemed to be withdrawn, only by the issue of such a sample to an expert nominated by the person requesting the sample.
(Rule 28(4) EPC).

C. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE [8] (if the indications are not for all designated States)

D. SEPARATE FURNISHING OF INDICATIONS [9] (leave blank if not applicable)

The indications listed below will be submitted to the International Bureau later [9] (Specify the general nature of the indications e.g., "Accession Number of Deposit")

E. [ ] This sheet was received with the international application when filed (to be checked by the receiving Office)

(Authorized Officer)

[ ] The date of receipt (from the applicant) by the International Bureau [10]

was

(Authorized Officer)

(January 1985)

CLAIMS

1. A polypeptide selected from the group comprising:

a) a polypeptide having the amino acid sequence of Formula I;
b) polypeptides having substantially the same structure and biological activity as a);
c) fragments, derivatives and mutants of a) or b) significantly involved in their biological activity;
and d) oligomeric forms of a), b) or c) having significant biological activity.

2. A polypeptide as claimed in claim 1 containing a conserved sequence as hereinbefore defined.

3. A polypeptide as claimed in claim 1 in which the sequence of amino acid residues is a conserved sequence as hereinbefore defined.

4. A fragment of the polypeptide of claim 1 in which the sequence of amino acid residues is selected from the region extending from residues 244-291 of Formula I.

5. A fragment as claimed in claim 4 in which the sequence of amino acids is selected from the group;

a) WDEWSPCSVTCGKGTRSRKR (residues 247 to 266)
b) WDEWSPCSVTCGKGTR (residues 249 to 260)
c) EWSPCSVTCGKG (residues 252 to 260)
d) PCSVTCGKG (residues 252 to 260)
e) WSPCSVTCG (residues 250 to 258)

6. A DNA sequence consisting essentially of a DNA sequence coding for a polypeptide as claimed in any one of the preceding claims.

7. A DNA sequence as claimed in claim 6 consisting essentially of the nucleotide coding sequence of Formula I (with or without the alterations displayed in Table I with reference to K1) or variations thereof not affecting the polypeptide encoded thereby or fragments or mutants thereof.

8. A recombinant vector containing a DNA sequence as claimed in claim 6 or 7.

9. A vector as claimed in claim 8 which is a plasmid having a molecular length of approximately 4.38 kb, and a restriction endonuclease map substantially as illustrated in Fig. 2.

10. A vector as claimed in claim 9 which is pKR5.

11. A vector as claimed in claim 8 which is a plasmid having a molecular length of approximately 11.5 kb, and a restriction endonuclease map substantially as illustrated in Fig. 3.

12. A vector as claimed in claim 11 which is pKKJ17 the plasmid deposited on 14th July, 1989 at the National Collections of Industrial and Marine Bacteria Ltd.(NCIMB), Torrey Research Station, P.O. Box 31, 135 Abbey Road, Aberdeen, AB9 8DG, United Kingdom, under Accession No. NCIMB 40,164.

13. A vector as claimed in claim 8 which is a recombinant virus or variant or mutant thereof.

14. A vector as claimed in claim 13 in which the virus is a baculovirus.

15. A vector as claimed in claim 14 in which the virus is Autographa californica Nucleopolyhedrosis Virus.

16. A vector as claimed in claim 15 in which the virus is Autographa californica Nucleopolyhedrosis Virus in conjunction with another vector as claimed in claim 11 or 12.

17. A viral vector as claimed in any one of claims 13 to 16 which is vKKJ17 or a variant or mutant thereof.

18. The virus deposited on 14th July, 1989 at European Collection of Animal Cell Cultures (ECACC), Public Health Service Laboratory, Centre for Applied Microbiology and Research, Porton Down, Salisbury, Wiltshire, SP4 OJG, United Kingdom, under Accession No. 89071402 or a variant or mutant thereof.

19. A host/vector system capable of expressing a DNA sequence as claimed in claims 6 or claim 7 and comprising a vector as claimed in any one of claims 8-18.

20. A host/vector system as claimed in claim 19 in which the host is selected from the species Spodoptera frugiperda.

21. A method for the preparation of a polypeptide as claimed in any one of claims 1-5 comprising the expression of a DNA sequence as claimed in claim 6 or claim 7 in a host/vector expression system.

22. A method as claimed in claim 21 which comprises the use of a vector as claimed in any one of claims 8-18.

23. A method as claimed in claim 21 which comprises the use of a host/vector system as claimed in claim 19 or claim 20.

24. A method of preparing a vector as claimed in anyone of claims 13-18 comprising the steps of:

a) preparing a plasmid containing a DNA sequence as

claimed in claim 6 or claim 7;

b) co-transfecting a host cell with DNA from the said sequence and from a virus;

c) recovering recombinant virus containing the said DNA sequence.

25. A method as claimed in claim 24 in which the plasmid is a vector as claimed in any one of claims 8 to 12.

26. A method as claimed in claim 24 or 25 in which the virus is a baculovirus.

27. A method as claimed in claim 26 in which the virus is the Autographa californica Nucleopolyhedrosis Virus.

28. A method as claimed in claim 27 in which the recombinant virus is vKKJ17 or a variant or mutant thereof.

29. A method as claimed in any one of claims 24-28 in which the host cell is of the species Spodoptera frugiperda.

30. An antibody capable of binding to a polypeptide as claimed in any one of claims 1-5.

31. An antibody as claimed in claim 30 having a paratope capable of binding to an epitope of the said polypeptides, which epitope is conserved throughout the said polypeptides.

32. An immunologically active composition comprising as an active constituent a polypeptide as claimed in any one of claims 1-5 in a pharmacologically acceptable vehicle.

33. A prophylactic or curative treatment against malaria comprising the administration of a composition as claimed in claim 37 in therapeutically effective dosage to a person suffering or at risk from the disease (USA only).

AMENDED CLAIMS

[received by the International Bureau on 15 January 1990 (15.01.90)
claims 1,4,5,7,32 and 33 amended; new claim 34 added; other claims unchanged (5 pages)]

1. A polypeptide selected from the group comprising:

   a) a polypeptide having the amino acid sequence of Formula I;
   b) polypeptides having substantially the same structure and biological activity as a);
   c) mutants of a) or b) significantly involved in their biological activity;
   d) oligomeric forms of a), b) or c) having significant biological activity;
   e) fragments of a), b), or c) significantly involved in their biological activity
   and derivatives of a), b), c), d), or e).

2. A polypeptide as claimed in claim 1 containing a conserved sequence as hereinbefore defined.

3. A polypeptide as claimed in claim 1 in which the sequence of amino acid residues is a conserved sequence as hereinbefore defined.

4. A polypeptide fragment as claimed in claim 1 in which the sequence of amino acid residues is selected from the region extending from residues 244-291 of Formula I.

5. A fragment as claimed in claim 4 in which the sequence of amino acids is selected from the group;

   a) WDEWSPCSVTCGKGTRSRKR (residues 247 to 266)
   b) WDEWSPCSVTCGKGTR (residues 247 to 262)
   c) EWSPCSVTCGKG (residues 249 to 260)
   d) PCSVTCGKG (residues 252 to 260)
   e) WSPCSVTCG (residues 250 to 258)

6. A DNA sequence consisting essentially of a DNA sequence coding for a polypeptide as claimed in any one of the preceding claims.

7. A DNA sequence as claimed in claim 6 consisting essentially of the nucleotide coding sequence of Formula I (with or without any of the alterations displayed in Table I with reference to K1) or variations thereof not affecting the polypeptide encoded thereby or fragments or mutants thereof.

8. A recombinant vector containing a DNA sequence as claimed in claim 6 or 7.

9. A vector as claimed in claim 8 which is a plasmid having a molecular length of approximately 4.38 kb, and a restriction endonuclease map substantially as illustrated in Fig. 2.

10. A vector as claimed in claim 9 which is pKR5.

11. A vector as claimed in claim 8 which is a plasmid having a molecular length of approximately 11.5 kb, and a restriction endonuclease map substantially as illustrated in Fig. 3.

12. A vector as claimed in claim 11 which is pKKJ17 the plasmid deposited on 14th July, 1989 at the National Collections of Industrial and Marine Bacteria Ltd.(NCIMB), Torrey Research Station, P.O. Box 31, 135 Abbey Road, Aberdeen, AB9 8DG, United Kingdom, under Accession No. NCIMB 40,164.

13. A vector as claimed in claim 8 which is a recombinant virus or variant or mutant thereof.

14. A vector as claimed in claim 13 in which the virus is a baculovirus.

15. A vector as claimed in claim 14 in which the virus is Autographa californica Nucleopolyhedrosis Virus.

16. A vector as claimed in claim 15 in which the virus is Autographa californica Nucleopolyhedrosis Virus in conjunction with another vector as claimed in claim 11 or 12.

17. A viral vector as claimed in any one of claims 13 to 16 which is vKKJ17 or a variant or mutant thereof.

18. The virus deposited on 14th July, 1989 at European Collection of Animal Cell Cultures (ECACC), Public Health Service Laboratory, Centre for Applied Microbiology and Research, Porton Down, Salisbury, Wiltshire, SP4 OJG, United Kingdom, under Accession No. 89071402 or a variant or mutant thereof.

19. A host/vector system capable of expressing a DNA sequence as claimed in claims 6 or claim 7 and comprising a vector as claimed in any one of claims 8-18.

20. A host/vector system as claimed in claim 19 in which the host is selected from the species Spodoptera frugiperda.

21. A method for the preparation of a polypeptide as claimed in any one of claims 1-5 comprising the expression of a DNA sequence as claimed in claim 6 or claim 7 in a host/vector expression system.

22. A method as claimed in claim 21 which comprises the use of a vector as claimed in any one of claims 8-18.

23. A method as claimed in claim 21 which comprises the use of a host/vector system as claimed in claim 19 or claim 20.

24. A method of preparing a vector as claimed in anyone of claims 13-18 comprising the steps of:

    a) preparing a plasmid containing a DNA sequence as claimed in claim 6 or claim 7;
    b) co-transfecting a host cell with DNA from the said sequence and from a virus;
    c) recovering recombinant virus containing the said DNA sequence.

25. A method as claimed in claim 24 in which the plasmid is a vector as claimed in any one of claims 8 to 12.

26. A method as claimed in claim 24 or 25 in which the virus is a baculovirus.

27. A method as claimed in claim 26 in which the virus is the Autographa californica Nucleopolyhedrosis Virus.

28. A method as claimed in claim 27 in which the recombinant virus is vKKJ17 or a variant or mutant thereof.

29. A method as claimed in any one of claims 24-28 in which the host cell is of the species Spodoptera frugiperda.

30. An antibody capable of binding to a polypeptide as claimed in any one of claims 1-5.

31. An antibody as claimed in claim 30 having a paratope capable of binding to an epitope of the said polypeptides, which epitope is conserved throughout the said polypeptides.

32. A pharmaceutical composition, for example an immunologically active composition such as a vaccine comprising as an active constituent a polypeptide as claimed in any one of claims 1-5 in a pharmacologically acceptable vehicle.

33. A prophylactic or curative treatment against malaria comprising the administration of a composition as claimed in claim 32 in therapeutically effective dosage to a person suffering or at risk from the disease (USA only).

34. A polypeptide derivative as claimed in claim 1 or claim 2 which is a fusion protein.

STATEMENT UNDER ARTICLE 19

The wording of claims 1, 4 and 7 has been clarified.

Claim 5 has been amended to correct obvious errors in the numbering of the residues. Internal comparisons within the claim and with Formula I of the specification are now consistent.

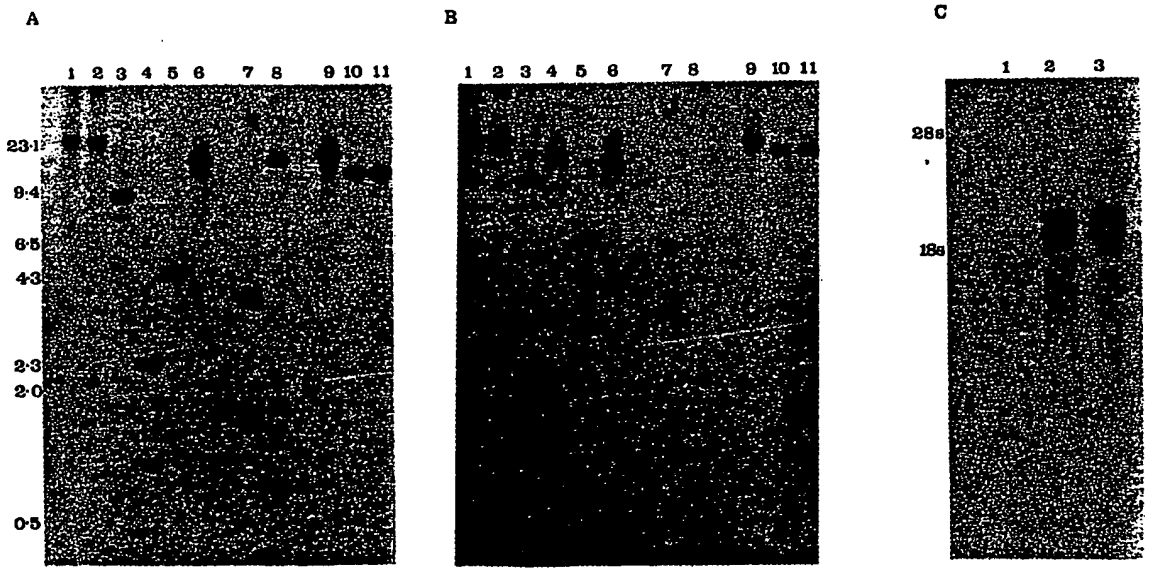
Claim 32 has been amended to clarify the meaning of the original terminology. The reference to a vaccine is supported by the disclosure at page 8 lines 30 to 32.

Claim 33 has been amended by the correction of an obvious error in that the reference to claim 37 was clearly intended to refer to claim 32.

New claim 34, to fusion proteins is supported by the disclosure at page 12 lines 13 and 14 and at page 17 line 8.

It is believed that these amendments will put the claims into better form for subsequent examination in the light of the search report.

Fig. 1.


A
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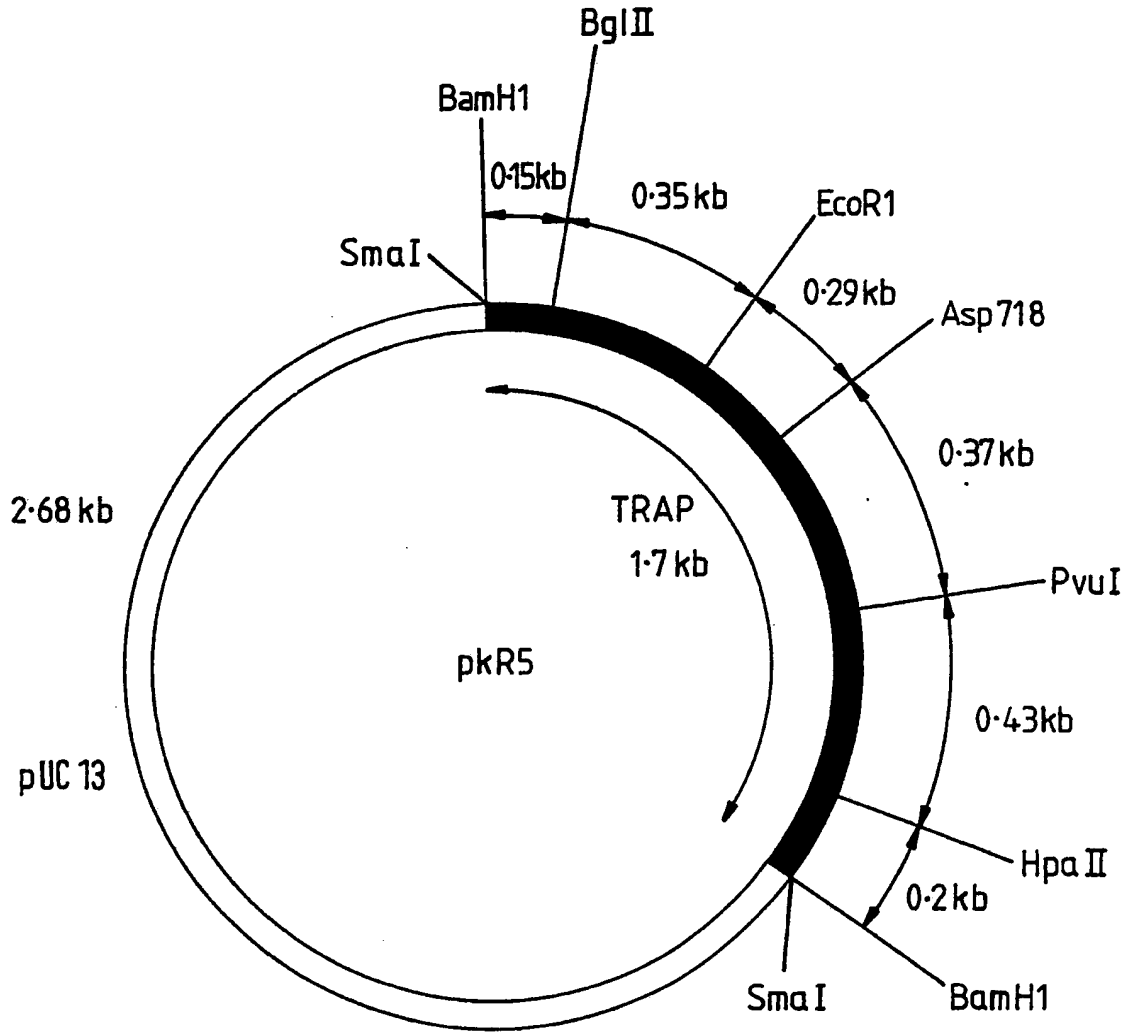
23·1
9·4
6·5
4·3
2·3
2·0
0·5
B
1 2 3 4 5 6 7 8 9 10 11
C
1 2 3
28s
18s

Fig. 2.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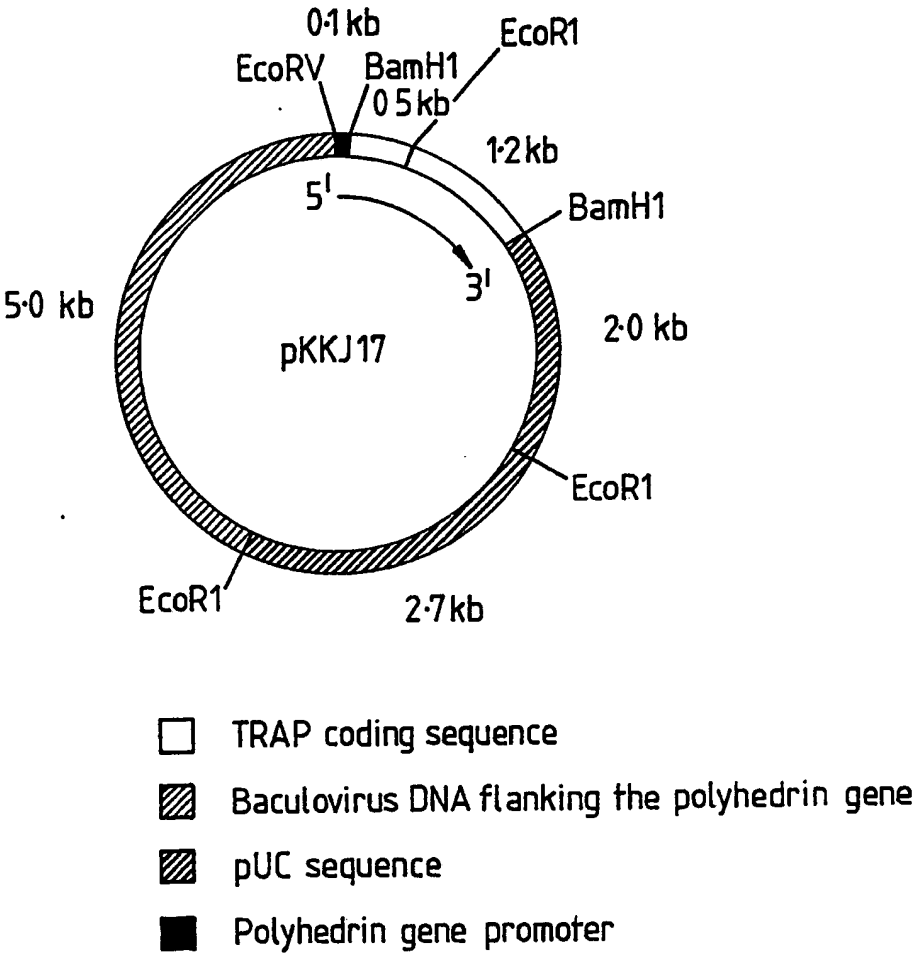
0·35kb
EcoR1
SmaI
0·29kb
Asp718
0·37kb
2·68kb
TRAP
1·7kb
PvuI
pkR5
0·43kb
pUC 13
HpaII
0·2kb
SmaI
BamH1

Fig. 3.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EcoRV
BamH1
EcoR1
0·5 kb
1·2 kb
5'
3'
BamH1
5·0 kb
pKKJ17
2·0 kb
EcoR1
EcoR1
2·7 kb
TRAP coding sequence
Baculovirus DNA flanking the polyhedrin gene
pUC sequence
Polyhedrin gene promoter

INTERNATIONAL SEARCH REPORT

International Application No PCT/GB 89/00895

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[5]: C 07 K 13/00, C 07 K 7/00, C 12 N 15/30, C 12 N 15/86, C 12 P 21/02, C 12 P 21/08, C 12 N 5/16, A 61 K 39/015

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[5]	C 12 N, C 12 P, A 61 K

Documentation Searched other than Minimum Documentation to the Extent that such Documents are included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category [10]	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
P,X	Nature, vo. 355, 1 September 1988 (Londen, GB) K.J.H. Robson et al.: "A highly conserved amino-acid sequence in thrombospondin, properdin and in proteins from sporozoites and blood stages of a human malaria parasite" pages 79-82, see the whole article	1-32
A	WO, A, 88/00595 (SAMARANE PTY, LTD) 28 January 1988	
A	WO, A, 87/07908 (THE WELLCOME FOUNDATION LTD et al.) 30 December 1987	
A	WO, A, 84/02917 (THE WALTER AND ELIZA HALL INSTITUTE OF MEDICAL RESEARCH) 2 August 1984, see claims	
A	Biotechnology, vol. 6, January 1988, New York (US) V.A. Luckow et al.: "Trends in the development of baculovirus expression	

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* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
16th November 1989	06. 12. 89
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	F.M. VRIJDAG

FURTHER INFORMATION CONTINUED FROM THE SECOND SHEET

vectors",
pages 47-55

V. [X] OBSERVATIONS WHERE CERTAIN CLAIMS WERE FOUND UNSEARCHABLE [1]

This International search report has not been established in respect of certain claims under Article 17(2) (a) for the following reasons:

1. [X] Claim numbers 33 because they relate to subject matter not required to be searched by this Authority, namely:

See PCT Rule 39.1.(iv)
Methods for treatment of the human or animal body by surgery or therapy, as well as diagnostic methods.

2. [ ] Claim numbers....., because they relate to parts of the International application that do not comply with the prescribed requirements to such an extent that no meaningful International search can be carried out, specifically:

3. [ ] Claim numbers....., because they are dependent claims and are not drafted in accordance with the second and third sentences of PCT Rule 6.4(a).

VI. [ ] OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING [2]

This International Searching Authority found multiple inventions in this International application as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International search report covers all searchable claims of the International application.

2. [ ] As only some of the required additional search fees were timely paid by the applicant, this International search report covers only those claims of the International application for which fees were paid, specifically claims:

3. [ ] No required additional search fees were timely paid by the applicant. Consequently, this International search report is restricted to the invention first mentioned in the claims; it is covered by claim numbers:

4. [ ] As all searchable claims could be searched without effort justifying an additional fee, the International Searching Authority did not invite payment of any additional fee.

Remark on Protest

[ ] The additional search fees were accompanied by applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. GB 8900895

SA 30559

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 28/11/89
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
WO-A- 8800595	28-01-88	EP-A-	0276262	03-08-88
		GB-A-	2200641	10-08-88
		JP-T-	1500828	23-03-89
		AU-A-	7699887	10-02-88
WO-A- 8707908	30-12-87	AU-A-	7453687	07-01-88
		AU-A-	7542287	12-01-88
		EP-A-	0250261	23-12-87
		JP-T-	63503512	22-12-88
WO-A- 8402917	02-08-84	AU-B-	569722	18-02-88
		AU-A-	2384284	09-08-84
		EP-A-	0134799	27-03-85
		GB-A,B	2143830	20-02-85
		JP-T-	60500478	11-04-85
		OA-A-	7823	20-11-86